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(71) Applicant: CHIRON CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US).

(72) Inventors: SHYAMALA, Venkatakrishna; 11430 Sun Valley Drive, Oakland, CA 94605 (US). KAVANAUGH, Michael, W.; 205 Laverene Avenue, Mill Valley, CA 94941 (US).

(74) Agents: POTTER, Jane, E., R. et al.; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US).

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(57) Abstract

A novel human gene encoding a protein termed MKK3-Interacting Protein (MIP) is disclosed. MIP expression is indicative of a tissue selected from the group of consisting of brain, kidney, liver, lung, pancreas, and spleen. MIP binds to a dominant interfering mutant of MAPK kinase-3 (MKK3) and may be involved in the transduction of extracellular signals into the nucleus which result in the activation of p38 kinase.

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(MITOGEN-ACTIVATED PROTEIN KINASE) KINASE-3(MKK3) INTERACTING PROTEIN (MIP)

TECHNICAL AREA OF THE INVENTION

The invention relates to the area of transduction of extracellular signals into the nucleus of human cells. More particularly, the invention relates to transduction of extracellular signals by means of the MKK3-p38 pathway.

BACKGROUND OF THE INVENTION

The mitogen-activated protein kinase (MAPK) cascade is a major signaling system by which cells transduce extracellular cues into intracellular responses. MAPKs phosphorylate substrates on serine or threonine adjacent to proline residues and are thus proline-directed protein kinases, as described in Cano and Mahadevan, *TIBS Reviews 20*:117-122 (1995). It is believed that multiple MAPK cascades exist, thus implicating many other molecules in the up and downstream events contributing to MAPK signal transduction events.

The p38 MAPK signal transduction pathway is activated by proinflammatory cytokines and environmental stress (Xia *et al, Science 270*: 1326-1331, 1995). p38 MAPK is weakly activated by protein kinase C and receptor tyrosine kinases but is strongly activated by the treatment of cells with inflammatory cytokines (including tumor necrosis factor and interleukin-1) and environmental stress (including osmotic shock and UV radiation), as described in Raingeaud *et al, Mol. Cell Biol 16*: 1247-1255 (1996).

MAPK kinase-3 (MKK3) is a protein kinase that phosphorylates and activates p38 MAP kinase specifically (Derijard *et al, Science 267*: 682-685, 1995). MKK3 is involved in transducing stress signals, for example in nerve growth factor (NGF)-mediated apoptosis in PC12 cells (Xia *et al, Science 270*: 1326-1331, 1995). MKK3 activity has also been correlated with changes in osmolarity.

The pathway responsible for transducing environmental stress signals in cells has not been fully described. Thus, there is a need in the art for the identification of proteins which are involved in the stress signal transduction pathway. Such proteins could be manipulated, for example, to protect cells against stress due to disease or environmental conditions.

SUMMARY OF THE INVENTION

It is an object of the invention to provide an isolated human protein which interacts with

MKK3.

It is another object of the invention to provide a fusion protein comprising such a human protein.

It is yet another object of the invention to provide an isolated polypeptide comprising epitopes of a MKK3 interacting protein.

It is still another object of the invention to provide a preparation of antibodies which specifically bind to a human protein which interacts with MKK3.

It is yet another object of the invention to provide a subgenomic polynucleotide which encodes all or a portion of a human protein which interacts with MKK3.

It is still another object of the invention to provide a method of determining the tissue source of a body sample of a human.

These and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention provides an isolated human MKK3-interacting protein (MIP). The MIP protein has the amino acid sequence shown in SEQ ID NO:1.

Another embodiment of the invention provides a MIP fusion protein. The fusion protein comprises a first protein segment and a second protein segment fused to each other by means of a peptide bond. The first protein segment consists of at least eight contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:1.

Yet another embodiment of the invention provides an isolated MIP polypeptide. The polypeptide consists of at least eight contiguous amino acids of a human MIP protein having the amino acid sequence shown in SEQ ID NO:1.

Still another embodiment of the invention provides a preparation of antibodies which specifically bind to a human MIP protein. The human MIP protein has the amino acid sequence shown in SEQ ID NO:1.

Even another embodiment of the invention provides a subgenomic polynucleotide which encodes all or a portion of a human MIP protein. The MIP protein has the amino acid sequence shown in SEQ ID NO:1.

Yet another embodiment of the invention provides a method of determining the tissue source of a body sample of a human. The method comprises the step of assaying the body sample for the presence of a human MIP protein or a 2.0 kb MIP mRNA encoding the MIP protein. The MIP protein has the amino acid sequence shown in SEQ ID NO:1. The presence of the human MIP protein or the 2.0 kb MIP mRNA encoding the MIP protein indicates that

the body sample originates from a tissue selected from the group consisting of brain, kidney, liver, lung, pancreas, and spleen.

The present invention provides the art with the amino acid sequence and DNA coding sequence of MIP, a unique human protein that binds to a dominant interfering mutant of MKK3. The present invention also provides the art with the information that MIP mRNA is differentially expressed in human tissues. The invention can be used, *inter alia*, to determine the tissue source of human body samples.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

It is a discovery of the present invention that MIP protein binds to and interacts with a dominant interfering mutant form of MKK3. Thus, MIP is likely involved in transducing extracellular signals to wild-type MKK3. It is also a discovery of the present invention that MIP mRNA is differentially expressed in human tissues.

Human MIP protein has the sequence disclosed in SEQ ID NO:1 and a molecular weight of approximately 40 kD. The protein is expressed in human brain, kidney, liver, lung, pancreas, and spleen, and not in heart or striated muscle. Any naturally occurring, biologically active variants of this sequence that occur in human tissues are within the scope of this invention. Naturally occurring, biologically active variants of MIP bind to a dominant interfering mutant of MKK3.

MIP protein is useful, *inter alia*, for generating antibodies against MIP protein sequences. Fragments (polypeptides) of a human MIP protein, comprising at least eight, ten, twelve, or fifteen consecutive amino acids selected from SEQ ID NO:1, may also be used as immunogens.

MIP protein may be isolated from MIP-producing human cells, such as brain, kidney, liver, lung, pancreas, or spleen cells. MIP may be obtained substantially free from other human proteins by standard protein purification methods, such as size exclusion chromatography, ion exchange chromatography, ammonium sulfate fractionation, affinity chromatography, or preparative gel electrophoresis. Alternatively, synthetic chemistry methods, such as solid-phase peptide synthesis, can be used to synthesize MIP protein or polypeptides. MIP protein or polypeptides may also be produced recombinantly, by expressing MIP coding sequences selected from SEQ ID NO:2 in prokaryotic or eukaryotic host cells, such as bacteria, yeast, insect, or mammalian cells, using expression vectors known in the art. Enzymes may be used to generate MIP polypeptides by enzymatic proteolysis of full-length

MIP protein.

MIP protein or polypeptides may also be used in a fusion protein, for example as an immunogen. The fusion protein comprises two protein segments. The first protein segment consists of at least 8, 10, 12, or 15 contiguous amino acids of MIP selected from the amino acid sequence shown in SEQ ID NO:1. The first protein segment is fused to a second protein segment by means of a peptide bond. The second protein segment may be a full-length protein or a fragment of a protein. Techniques for making fusion proteins, either recombinantly or by covalently linking two protein segments, are well known in the art. The second protein or protein fragment may be, for example, a ligand for yet a third molecule. The second protein or protein fragment may be labeled with a detectable marker, such as a radioactive or fluorescent tag, or may be an enzyme that will generate a detectable product. Enzymes suitable for this purpose, such as β-galactosidase, are well-known in the art. A fusion protein may be used, for example, to target MIP protein or MIP polypeptides to a particular location in a cell or tissue, to use MIP protein or polypeptides in various assays, such as the yeast two-hybrid technique, or as an immunogen.

MIP proteins, fusion proteins, or polypeptides may be used to obtain a preparation of antibodies that specifically bind to a human MIP protein. The antibodies may be polyclonal or monoclonal. Techniques for raising both polyclonal and monoclonal antibodies are well known in the art. The antibodies bind specifically to MIP epitopes. In a preferred embodiment, the MIP epitopes are not present on other human proteins. Typically a minimum number of contiguous amino acids to encode an epitope is 6, 8, or 10. However, more may be used, for example, at least 15, 25, or 50, especially to form epitopes which involve non-contiguous residues.

Antibodies that bind specifically to MIP proteins include those that bind to full-length MIP protein, MIP polypeptides, or MIP fusion proteins. Specific binding antibodies do not detect other proteins on Western blots of human cells, or provide a signal at least ten-fold lower than the signal provided with MIP. Most preferably the antibodies bind to neither GMP reductase nor glucose-6-phosphate dehydrogenase. Antibodies which have such specificity can be obtained by routine screening. In a preferred embodiment of the invention, the antibodies prevent MIP binding to a dominant interfering mutant of MKK3, immunoprecipitate MIP bound to the mutant MKK3 from a cell lysate, or react with MIP protein in tissue sections or on Western blots of polyacrylamide gels. Preferably the antibodies do not exhibit nonspecific cross-reactivity with other human proteins on Western blots or in immunocytochemical assays.

Techniques for purifying MIP antibodies are available in the art. In a preferred embodiment, antibodies are affinity purified by passing antiserum over a column to which a MIP protein, polypeptide, or fusion protein is bound and then eluting the bound antibody, for example with a buffer having a high salt concentration.

Subgenomic polynucleotides may encode all or a contiguous portion of MIP selected from the amino acid sequence of SEQ ID NO:1. The MIP coding sequence (cDNA) has the nucleotide sequence shown in SEQ ID NO:2. Any naturally occurring variants of this coding sequence which encode the amino acid sequence shown in SEQ ID NO:1 or polymorphic variants thereof are within the scope of this invention. The polynucleotides may be used to produce MIP protein, polypeptides, or fusion proteins, and may be used as probes for the detection of MIP mRNA in samples or extracts of human cells.

MIP subgenomic polynucleotides contain less than a whole chromosome and are preferably intron-free. MIP polynucleotides may be isolated and purified free from other nucleotide sequences by standard nucleic acid purification techniques, using restriction enzymes and probes to isolate fragments comprising the MIP encoding sequences. In a preferred embodiment, the polynucleotide molecules comprise a contiguous sequence of 12, 15, 20, 25, or 30 nucleotides selected from SEQ ID NO:2. MIP cDNA can be made using reverse transcriptase with MIP mRNA as a template. Amplification by PCR may also be used to obtain the polynucleotides, using either genomic DNA or cDNA as a template. Given the nucleotide sequence disclosed herein, the polynucleotide molecules can also be made using the techniques of synthetic chemistry. The degeneracy of the genetic code permits alternate nucleotide sequences to be synthesized that will encode the MIP amino acid sequence shown in SEQ ID NO:1. All such nucleotide sequences are within the scope of the present invention.

The MIP polynucleotides can be propagated in vectors and cell lines using techniques well known in the art. Expression systems in bacteria include those described in Chang *et al.*, *Nature* (1978) *275*: 615, Goeddel *et al.*, *Nature* (1979) *281*: 544, Goeddel *et al.*, *Nucleic Acids Res.* (1980) *8*: 4057, EP 36,776, U.S. 4,551,433, deBoer *et al.*, *Proc. Natl. Acad. Sci. USA* (1983) *80*: 21-25, and Siebenlist *et al.*, *Cell* (1980) *20*: 269. Expression systems in yeast include those described in Hinnen *et al.*, *Proc. Natl. Acad. Sci. USA* (1978) *75*: 1929; Ito *et al.*, *J. Bacteriol.* (1983) *153*: 163; Kurtz *et al.*, *Mol. Cell. Biol.* (1986) *6*: 142; Kunze *et al.*, *J. Basic Microbiol.* (1985) *25*: 141; Gleeson *et al.*, *J. Gen. Microbiol.* (1986) *132*: 3459, Roggenkamp *et al.*, *Mol. Gen. Genet.* (1986) *202* :302) Das *et al.*, *J. Bacteriol.* (1984) *158*: 1165; De Louvencourt *et al.*, *J. Bacteriol.* (1983) *154*: 737, Van den Berg *et al.*,

Bio/Technology (1990) *8*: 135; Kunze *et al.*, *J. Basic Microbiol.* (1985) *25*: 141; Cregg *et al.*, *Mol. Cell. Biol.* (1985) *5*: 3376, U.S. 4,837,148, US 4,929,555; Beach and Nurse, *Nature* (1981) *300*: 706; Davidow *et al.*, *Curr. Genet.* (1985) *10*: 380, Gaillardin *et al.*, *Curr. Genet.* (1985) *10*: 49, Ballance *et al.*, *Biochem. Biophys. Res. Commun.* (1983) *112*: 284-289; Tilburn *et al.*, *Gene* (1983) *26*: 205-221, Yelton *et al.*, *Proc. Natl. Acad. Sci. USA* (1984) *81*: 1470-1474, Kelly and Hynes, *EMBO J.* (1985) *4*: 475479; EP 244,234, and WO 91/00357. Expression of heterologous genes in insects can be accomplished as described in U.S. 4,745,051, Friesen *et al.* (1986) "The Regulation of Baculovirus Gene Expression" in: THE MOLECULAR BIOLOGY OF BACULOVIRUSES (W. Doerfler, ed.), EP 127,839, EP 155,476, and Vlak *et al.*, *J. Gen. Virol.* (1988) *69*: 765-776, Miller *et al.*, *Ann. Rev. Microbiol.* (1988) *42*: 177, Carbonell *et al.*, *Gene* (1988) *73*: 409, Maeda *et al.*, *Nature* (1985) *315*: 592-594, Lebacq-Verheyden *et al.*, *Mol. Cell. Biol.* (1988) *8*: 3129; Smith *et al.*, *Proc. Natl. Acad. Sci. USA* (1985) *82*: 8404, Miyajima *et al.*, *Gene* (1987) *58*: 273; and Martin *et al.*, *DNA* (1988) *7*:99. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts are described in Luckow *et al.*, *Bio/Technology* (1988) *6:* 47-55, Miller *et al.*, in GENETIC ENGINEERING (Setlow, J.K. *et al.* eds.), Vol. 8 (Plenum Publishing, 1986), pp. 277-279, and Maeda *et al.*, *Nature*, (1985) *315*: 592-594. Mammalian expression can be accomplished as described in Dijkema *et al.*, *EMBO J.* (1985) *4*: 761, Gorman *et al.*, *Proc. Natl. Acad. Sci. USA* (1982b) *79*: 6777, Boshart *et al.*, *Cell* (1985) *41*: 521 and U.S. 4,399,216. Other features of mammalian expression can be facilitated as described in Ham and Wallace, *Meth. Enz.* (1979) *58*: 44, Barnes and Sato, *Anal. Biochem.* (1980) *102*: 255, U.S. 4,767,704, US 4,657,866, US 4,927,762, US 4,560,655, WO 90/103430, WO 87/00195, and U.S. RE 30,985.

The MIP polynucleotides may be on linear or circular molecules. They may be on autonomously replicating molecules or on molecules without replication sequences. They can be regulated by their own or by other regulatory sequences as are known in the art. MIP polynucleotides may be introduced into suitable host cells using a variety of techniques which are available in the art, such as transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated DNA transfer, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, and calcium phosphate-mediated transfection.

The present invention also provides a method of determining the tissue source of a human body sample. The basis for this method is the discovery that MIP mRNA is expressed

in some human tissues, such as brain, kidney, liver, lung, pancreas, and spleen, but not in others, such as heart or striated muscle.

In one embodiment, the body sample is assayed for the presence of a human MIP protein. The MIP protein has the sequence shown in SEQ ID NO:1 and may be detected using the MIP-specific antibodies of the present invention. The antibodies may be labeled, for example, with a radioactive, fluorescent, biotinylated, or enzymatic tag and detected directly, or may be detected using indirect immunochemical methods, using a labeled secondary antibody. The presence of MIP protein may be assayed in tissue sections by immunocytochemistry, or in Western blots of lysates.

In another embodiment, the body sample is assayed for the presence of MIP mRNA. MIP mRNA is 2.0 kb in length and may be detected by *in situ* hybridization in tissue sections or in Northern blots containing poly A+ mRNA. MIP-specific probes may be generated using the MIP cDNA sequence disclosed herein (SEQ ID NO:2). The probes are preferably 15 to 50 nucleotides in length, although they may be 8, 10, 20, 25, 30, 35, 40, 45, 60, 75, or 100 nucleotides in length. The probes may be synthesized chemically or may be generated from longer polynucleotides using restriction enzymes. The probes may be labeled, for example, with a radioactive, biotinylated, or fluorescent tag.

The body sample which is assayed may be normal or may be diseased. In a preferred embodiment, the body sample is a primary tumor or a metastatic lesion. Metastatic lesions originating from tumors of the brain, kidney, liver, lung, pancreas, and spleen may be identified using this method. Other tissues can also be tested for the presence or absence of MIP protein or mRNA.

The following are provided for exemplification purposes only and are not intended to limit the scope of the invention which has been described in broad terms above.

EXAMPLE 1

This example demonstrates the identification of MIP cDNA.

All general yeast protocols in this example are described in Methods in Enzymology, vol. 194, "Guide to Yeast Genetics and Molecular Biology," by Guthrie and Fink. Ausubel *et al* (1994) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, (Greene Publishing Associates and John Wiley & Sons, New York, NY), and Sambrook *et al.* (1989), MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed. (Cold Spring Harbor Press, Cold Spring Harbor, New York), are appropriate general references.

The yeast 2-hybrid system, as described in U.S. Patent No. 5,283,173 and Chien *et al*, *Proc. Nat'l Acad Sci USA 88*:9578-9582 (1991), was used to identify a protein which interacts with a dominant interfering mutant of MKK3. MKK3 wild type was obtained by RT-PCR from human brain poly A+ RNA. A dominant interfering mutant of MKK3 (K-R) was constructed using overlap PCR, as described in Shyamala and Asmes, *Gene 97*: 1-6 (1991). Fragments which contained a sequence differing from the wild-type sequence by amino acid K64R were cloned into a pAS vector and used as bait DNA. The pAS vector is yeast expression vector with a Gal4 binding domain and other required features.

The bait DNA along with a human fetal brain cDNA library in a pAD-Gal4 vector (Stratagene, La Jolla, CA) was used for transformation. The pAD-Gal4 vector has the Gal4 activation domain and other required features.

Fifty μg of the bait DNA in the pAS vector and 50 μg of library DNA in pAD vector was used to transform 5.0 ml of competent yeast cells (Stratagene, La Jolla, CA). Autoactivation was tested by transforming 0.1 ml of yeast cells with 1.0 μg of bait in the pAS vector alone.

A total of 1×10^6 transformants were obtained for both wild type and mutant MKK3 using the library DNA. In selective media, 5×10^4 and 5×10^5 colonies were obtained for the MKK3 wild type and mutant, respectively.

The colonies were examined for the expression of β-galactosidase activity by filter lifts, as described by Clontech, Palo Alto, CA. Six colonies were positive using the wild type MKK3, and ten colonies were positive using the dominant interfering mutant bait. All positive colonies were recultured on selection plates. Upon sufficient growth of the yeast colony, DNA was isolated as described by Stratagene, La Jolla, CA. An aliquot was used to perform PCR with (a) MKK3 DNA specific primers to confirm the presence of the bait plasmid and (b) pAD vector specific primers bracketing the insert to confirm presence of the insert. All of the clones were positive for both plasmids. Another aliquot was used for transforming bacterial cells. Plasmid DNA was then isolated and sequenced.

The pAD vector containing the MKK3-interacting protein (MIP) when used for transformation did not result in β-galactosidase-positive colonies. Three of the MKK3 mutant interacting clones, however, contained an identical sequence with a unique open reading frame of 348 amino acids and 337 amino acids as a fusion with Gal4 activation protein. None of the other clones interacting with the MKK3 wild type or the mutant contained a considerable open reading frame.

Thus, MIP binds to and interacts with a dominant interfering mutant form of MKK3.

EXAMPLE 2

This example demonstrates the isolation and cloning of the 5' end of the human MIP cDNA.

The 5' end of the MKK3 interacting protein (MIP) cDNA was isolated by performing RT-PCR on brain and liver poly A+ RNA. Sequence of the PCR fragments indicated 190 nucleotides at the 5' end, with a possible initiating methionine at nucleotide 167. A Kozak consensus sequence of GCGCC was present 5' to the methionine.

EXAMPLE 3

This example demonstrates that MIP is localized to chromosome 14.

Chromosome localization of MIP was determined by performing PCR with MIP-specific primers on DNA from mouse human hybrids (BIOS Labs, New Haven, CT). The MIP gene was localized to chromosome 14.

EXAMPLE 4

This example demonstrates the molecular weight of MIP protein.

A flag epitope tagged MIP was constructed in a pCMV-flag vector (cytomegalovirus vector with a sequence coding the 8 amino acid flag polypeptide tag; Chubet and Brizzard, *Bio/Techniques 20*: 136, 1996). The tagged MIP protein was then expressed in host cells. Protein from the host cells was extracted and fractionated electrophoretically. An anti-flag antibody detected a protein of about 40 kD on film from the protein gel. Thus, MIP protein has a molecular weight of about 40 kD.

EXAMPLE 5

This example demonstrates the tissue-specific expression of MIP mRNA.

MIP mRNA was detected in a blot of poly A+ mRNA from various normal adult human tissues using a MIP cDNA probe. A hybridization signal corresponding to a 2.0 kb mRNA was visualized in the brain (moderately strong), kidney (moderately strong), liver (moderate), lung (weak), pancreas (moderate), and spleen (very strong). No hybridization was detected in heart or striated muscle.

Thus, MIP mRNA is differentially expressed in adult human tissues.

DEPOSIT INFORMATION

The following material was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 20852, under the terms of the Budapest Treaty:

Name	Deposit Date	Accession No.
pFLAG-MIP	27 June 1996	97638

pFLAG-MIP is a DNA plasmid that is transformed into DH5α cells. The vector contains an ampicillin marker, and the insert can be excised with EcoRI and SmaI restriction enzymes. The plasmid is 6130 base pairs, and the insert containing the coding region for MIP is 1053 base pairs.

These deposits are provided merely as convenience to those of skill in the art, and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained within the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated by reference and are controlling in the event of any conflict with the written description of sequences herein. A license may be required to make, use, or sell the deposited materials, and no such license is granted hereby.

SYNOPSIS OF THE INVENTION

1. An isolated human MIP protein having the amino acid sequence shown in SEQ ID NO:1.

2. A MIP fusion protein which comprises a first protein segment and a second protein segment fused to each other by means of a peptide bond, wherein the first protein segment consists of at least eight contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:1.

3. An isolated MIP polypeptide which consists of at least eight contiguous amino acids of a human MIP protein having the amino acid sequence shown in SEQ ID NO:1.

4. A preparation of antibodies which specifically bind to a human MIP protein with the amino acid sequence shown in SEQ ID NO:1.

5. The preparation of antibodies of item 4 wherein the antibodies are monoclonal.

6. The preparation of antibodies of item 4 wherein the antibodies are polyclonal.

7. The preparation of antibodies of item 4 wherein the antibodies are affinity purified.

8. A subgenomic polynucleotide which encodes at least eight contiguous amino acids of a human MIP protein having the amino acid sequence shown in SEQ ID NO:1.

9. The subgenomic polynucleotide of item 8 having the nucleotide sequence shown in SEQ ID NO:2.

10. The subgenomic polynucleotide of item 8 which is intron-free.

11. A method of determining the tissue source of a body sample of a human, comprising the step of:

assaying the body sample for the presence of a human MIP protein having the amino acid sequence shown in SEQ ID NO:1 or a 2.0 MIP mRNA encoding said human MIP protein, the presence of said human MIP protein or said 2.0 kb MIP mRNA encoding said human MIP protein indicating that the body sample originates from a tissue selected from the group consisting of brain, kidney, liver, lung, pancreas, and spleen.

12. The method of item 11 wherein the body sample is a tumor.

13. The method of item 11 wherein the body sample is a metastatic lesion.

14. The method of item 11 wherein the step of assaying is immunohistochemical.

15. The method of item 11 wherein the step of assaying employs nucleic acid hybridization.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: CHIRON CORPORATION

(ii) TITLE OF INVENTION: MKK3-Interacting Protein (MIP)

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Chiron Corporation
(B) STREET: Intellectual Property - P.O. Box 8097
(C) CITY: Emeryville
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94662-8097

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Unassigned
(B) FILING DATE: Even date herewith
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Jane Potter
(B) REGISTRATION NUMBER: 33,332
(C) REFERENCE/DOCKET NUMBER: 0125.100

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (510) 923-2719
(B) TELEFAX: (510) 655-3542

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 348 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Pro His Ile Asp Asn Asp Val Lys Leu Asp Phe Lys Asp Val Leu
1               5                  10                  15

Leu Arg Pro Lys Arg Ser Thr Leu Lys Ser Arg Ser Glu Val Asp Leu
            20                  25                  30

Thr Arg Ser Phe Ser Phe Arg Asn Ser Lys Gln Thr Tyr Ser Gly Val
        35                  40                  45
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Pro Ile Ile Ala Ala Asn Met Asp Thr Val Gly Thr Phe Glu Met Ala
    50                  55                  60

Lys Val Leu Cys Lys Phe Ser Leu Phe Thr Ala Val His Lys His Tyr
65                  70                  75                  80

Ser Leu Val Gln Trp Gln Glu Phe Ala Gly Gln Asn Pro Asp Cys Leu
                85                  90                  95

Glu His Leu Ala Ala Ser Ser Gly Thr Gly Ser Ser Asp Phe Glu Gln
            100                 105                 110

Leu Glu Gln Ile Leu Glu Ala Ile Pro Gln Val Lys Tyr Ile Cys Leu
        115                 120                 125

Asp Val Ala Asn Gly Tyr Ser Glu His Phe Val Glu Phe Val Lys Asp
    130                 135                 140

Val Arg Lys Arg Phe Pro Gln His Thr Ile Met Ala Gly Asn Val Val
145                 150                 155                 160

Thr Gly Glu Met Val Glu Glu Leu Ile Leu Ser Gly Ala Asp Ile Ile
                165                 170                 175

Lys Val Gly Ile Gly Pro Gly Ser Val Cys Thr Thr Arg Lys Lys Thr
            180                 185                 190

Gly Val Gly Tyr Pro Gln Leu Ser Ala Val Met Glu Cys Ala Asp Ala
        195                 200                 205

Ala His Gly Leu Lys Gly His Ile Ile Ser Asp Gly Gly Cys Ser Cys
    210                 215                 220

Pro Gly Asp Val Ala Lys Ala Phe Gly Ala Gly Ala Asp Phe Val Met
225                 230                 235                 240

Leu Gly Gly Met Leu Ala Gly His Ser Glu Ser Gly Gly Glu Leu Ile
                245                 250                 255

Glu Arg Asp Gly Lys Lys Tyr Lys Leu Phe Tyr Gly Met Ser Ser Glu
            260                 265                 270

Met Ala Met Lys Lys Tyr Ala Gly Gly Val Ala Glu Tyr Arg Ala Ser
        275                 280                 285

Glu Gly Lys Thr Val Glu Val Pro Phe Lys Gly Asp Val Glu His Thr
    290                 295                 300

Ile Arg Asp Ile Leu Gly Gly Ile Arg Ser Thr Cys Thr Tyr Val Gly
305                 310                 315                 320

Ala Ala Lys Leu Lys Glu Leu Ser Arg Arg Thr Thr Phe Ile Arg Val
                325                 330                 335

Thr Gln Gln Val Asn Pro Ile Phe Ser Glu Ala Cys
            340                 345
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1698 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AGTGTGATGG ATATCTGCAG AATTCGGCTT TATTTAGGTG ACACTATAGA AGGTACGCCT 60

GCAGGTACCG GTCCGGAATT CCCGGGTCGA CCCACGCGTC CGGCAGGGGT AGAAGAAGGA 120

AGTGTAGCGG GCCCTCAGAT TCATCGCTAC CCCGAGGCTA AGCGCCATGC CTCATATTGA 180

CAACGATGTG AAACTGGACT TCAAGGATGT CCTTTTGAGG CCCAAACGCA GTACCCTTAA 240

GTCTCGAAGT GAGGTGGATC TCACAAGATC CTTTTCATTT CGGAACTCAA AGCAGACATA 300

CTCTGGGGTT CCCATCATTG CTGCCAATAT GGATACTGTG GGCACCTTTG AGATGGCCAA 360

GGTTCTCTGT AAGTTCTCTC TCTTCACTGC TGTCCATAAG CACTATAGCC TCGTTCAGTG 420

GCAAGAGTTT GCTGGCCAGA ATCCTGACTG TCTTGAGCAT CTGGCTGCCA GCTCAGGCAC 480

AGGCTCTTCT GACTTTGAGC AGCTGGAACA GATCCTGGAA GCTATTCCCC AGGTGAAGTA 540

TATATGCCTG GATGTGGCAA ATGGCTACTC TGAACACTTT GTTGAATTTG TAAAAGATGT 600

ACGGAAGCGC TTCCCCCAGC ACACCATCAT GGCAGGGAAT GTGGTAACAG GAGAGATGGT 660

AGAAGAGCTC ATCCTTTCTG GGGCTGACAT CATCAAAGTG GGAATTGGGC CAGGCTCTGT 720

GTGTACTACT CGGAAGAAAA CTGGAGTGGG GTATCCACAG CTCAGCGCAG TGATGGAGTG 780

TGCAGATGCT GCTCATGGCC TCAAAGGCCA CATCATTTCA GATGGAGGTT GCAGCTGTCC 840

TGGGGATGTG GCCAAGGCTT TTGGGGCAGG AGCTGACTTC GTGATGCTGG GTGGCATGCT 900

GGCTGGGCAC AGTGAGTCAG GTGGTGAGCT CATCGAGAGG GATGGCAAGA AGTACAAGCT 960

CTTCTATGGA ATGAGTTCTG AAATGGCCAT GAAGAAGTAT GCTGGGGGCG TGGCTGAGTA 1020

CAGAGCCTCA GAGGGAAAGA CAGTGGAAGT TCCTTTTAAA GGAGATGTGG AACATACCAT 1080

CCGAGACATC CTAGGAGGGA TCCGCTCTAC GTGTACCTAT GTGGGAGCAG CTAAGCTCAA 1140

AGAGTTGAGC AGGAGAACTA CCTTCATCCG AGTCACCCAG CAGGTGAATC CAATCTTCAG 1200

TGAGGCGTGC TAGACCTGAG CAGTTCTACC CTCCCAAGGC ACCAGTACTC TACCATGGGG 1260

CATCCCAAGT GGGGTCCTCA CCCATCCCAG CTACTGCAGC TCTGTATTAC TTTGTCATTT 1320

CCTGTTGTCT CACTCCTGAG GGCTCCTGCA GTAACTCTGT ACTTCTCTAT CTGCACACAC 1380

AAAATGCCCA AGGCACTCAC TGGGGAGGAA GCAAGGAAGC AAACAGTCTG AGAAAATGAT 1440

GCAAGAAAAT CAAATGGGAA TCTGGGGACC CAACACAACA TCCTGAAGAT TATTAAAAGG 1500

AAAAGATGCT GATTGGTACA TAAATCTTTT ACATGGCCTT GGTCTAGAGG AGGCAGGCTT 1560

TTAGAATCAT GTTTTGTTAA TCCGCTTCAC TAAATTGGAC CTTCACATAT CTAAAAAGCT 1620

CTGAAGTGTT TGTATATTTG AAATACCTCA ATAAAGAGAG AGCTCATTGA CTGTAAAAAA 1680

AAAAAAAAAA AACTCGAG 1698

CLAIMS

1. An isolated human MIP protein having the amino acid sequence shown in SEQ ID NO:1.

2. A MIP fusion protein which comprises a first protein segment and a second protein segment fused to each other by means of a peptide bond, wherein the first protein segment consists of at least eight contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:1.

3. An isolated MIP polypeptide which consists of at least eight contiguous amino acids of a human MIP protein having the amino acid sequence shown in SEQ ID NO:1.

4. A preparation of antibodies which specifically bind to a human MIP protein with the amino acid sequence shown in SEQ ID NO:1.

5. A subgenomic polynucleotide which encodes at least eight contiguous amino acids of a human MIP protein having the amino acid sequence shown in SEQ ID NO:1.

6. A method of determining the tissue source of a body sample of a human, comprising the step of:

assaying the body sample for the presence of a human MIP protein having the amino acid sequence shown in SEQ ID NO:1 or a 2.0 MIP mRNA encoding said human MIP protein, the presence of said human MIP protein or said 2.0 kb MIP mRNA encoding said human MIP protein indicating that the body sample originates from a tissue selected from the group consisting of brain, kidney, liver, lung, pancreas, and spleen.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/10866

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 C07K16/18 G01N33/68 C12N15/62
C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	H. KANNO ET AL.: "Two structural genes on different chromosomes are required for encoding the major subunit of human cell glucose-6-phosphate dehydrogenase." CELL, vol. 58, 11 August 1989, pages 595-606, XP002054262 see the whole document ---	3,5
X	S. HENIKOFF ET AL.: "The human mRNA that provides the N-terminus of chimeric G6PD encodes GMP reductase." CELL, vol. 58, 22 September 1989, pages 1021-1022, XP002054263 see the whole document ---	3,5

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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 February 1998	19/02/1998
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Hix, R

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INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 97/10866

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	T. KONDOH ET AL.: "Genomic structure and expression of human guanosine monophosphate reductase." HUMAN GENETICS, vol. 88, 1991, pages 219-224, XP002054264 see the whole document	3,5
A	B. DÉRIJARD ET AL.: "Independent Human MAP kinase signal transduction pathways defined by MEK and MKK isoforms." SCIENCE, vol. 267, 3 February 1995, pages 682-685, XP002054336 cited in the application see the whole document	
A	Z. XIA ET AL.: "Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis." SCIENCE, vol. 270, 24 November 1995, pages 1326-1331, XP002054337 cited in the application see the whole document	